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INHIBITION OF RNA SYNTHESIS BY ACTINOMYCIN

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On the basis of simple statistical concepts, a study has been made of the mechanism of inhibition of RNA synthesis in a cell-free system. It has been demonstrated experimentally that the derived quantitative relationships can be used also in the case of intact cells. An estimate is given for the number of guanine-cytosine pairs in the considered DNA section. Under the influence of AM there first will be an inhibition of those RNA molecules which have the greatest molecular weight and whose nucleotide composition includes a relatively large number of guanine-cytosine pairs, that is, high-molecular RNA of the guanine-cytosine type. This also can be attributed to the fact that AM first inhibits synthesis of ribosomal RNA (high-molecular and guanine-cytosine type), and, second, inhibits the synthesis of messenger RNA (high-molecular, but the AU type) and, thereafter, inhibits the synthesis of transport RNA (low-molecular, but of the guanine-cytosine type).

The extensive use of actinomycin (AM) in cytological and biochemical investigations [1] makes it timely to analyze the problem of the applicability of statistical concepts to the mechanism of inhibition of the synthesis of RNA both in a cell-free system and within an intact cell. The inhibition of the synthesis of RNA by AM is governed by the effect of AM on the DNA template [2]. A complex of AM and DNA is formed [3]. A necessary condition for the formation of a complex of AM with DNA is the presence of guanine in the DNA [4]. /521*

Inhibition of RNA Synthesis in a Cell-Free System.

We will consider the following simple model of the suppression of the synthesis of RNA. Assume that in the system there is a single isolated DNA molecule which in the general complex contains N_0 guanine bases. Also assume that under the given conditions this molecule has an isolated functioning part which contains N guanine bases, and:

$$N_0 \gg N$$

Assuming that the AM molecules are bonded exclusively to the guanine bases of the DNA, the value N_0 may be considered the number of vacant places in the

*Numbers in the margin indicate pagination in the original foreign text.

complex. We also will assume that the joining of one molecule of AM is sufficient (appearance of one "clamp" on the DNA spiral) in order to inhibit removal of the DNA from this part [5,6]. In other words, it is assumed that for inhibition of RNA synthesis under these conditions, it is sufficient for one AM molecule to be attached to the functioning part (naturally, this does not prevent the attachment of other AM molecules on other guanine bases of the functioning or inactive part).

If one AM molecule is present in the system, on the assumption of equal accessibility of all the parts, the probability of the bonding of this molecule to the functioning part is equal to the ratio N/N_0 and the probability that this event will not occur (and, therefore, inhibition does not set in) will be equal to:

$$W_1 = 1 - N/N_0$$

If there are n AM molecules in the system, the probability of continuation of activity is defined by the equation:

$$W_n = (1 - N/N_0)^n \approx e^{-\frac{n}{N_0} \cdot N}$$

Finally, if the system contains a large number of identical DNA molecules which are in the same state, and as before there are n AM molecules for each DNA molecule, the value W_n determines the fraction of uninhibited DNA molecules, that is, the residual activity J of the system to RNA synthesis. Denoting the relative concentration of AM molecules per one guanine base by /522

$$c = n/N_0$$

we obtain:

$$J = J_0 e^{-Nc} \qquad (1)$$

In a somewhat more general case, when there are several, rather than a single functioning part, and these have different contents of guanine bases

$$N_1, N_2, \ldots N_i$$

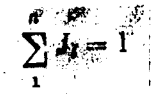
and they have a different intensity of synthesis

$$J_1, J_2, \ldots$$

the residual activity after inhibition by actinomycin can be expressed by the formula

(2)

Here, as before, the AM concentration is determined as the ratio of the number of AM molecules to the number of guanine bases; in addition, it is assumed that the following condition is satisfied:

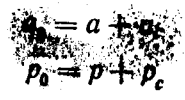


In formulas (1) and (2), the residual activity of DNA is expressed through the value c, that is, in the last analysis through the number of AM molecules bonded to the guanine bases. In addition, in actuality in an experiment we measure the total number of AM molecules introduced into the solution and the value

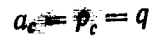
should be determined from the conditions of equilibrium in the reaction of formation of the AM-DNA complex, which can be written in the form:

$$AM + DNA \rightleftarrows (AM - DNA) \qquad (3)$$

We denote the initial concentration of AM molecules by a_o, and the concentration of guanine bases by p_o. The concentration of AM molecules in a free state is denoted by a, in the bound state -- by a_c, the number of free places -- by p, the occupied spaces -- by p_c. Then we can write:



In addition, obviously:



Therefore, the relative concentration will be equal to:

$$c = n/N_o = q/p_o \text{ mole AM/mole G(uanine)}$$

Under conditions of equilibrium the law of ~~active masses~~ mass action gives:

$$K = \frac{q}{a \cdot p},$$

where K is the equilibrium constant. Then: /523

$$K = \frac{q}{p(a_0 - q)}$$

If it is assumed that the concentration of free nucleotides is much greater than the concentration of ~~bonded~~ bound nucleotides, that is $p_o \gg q$, then

$$K = \frac{q}{p_0(a_0 - q)}$$

or

$$\frac{q}{p_0} = \frac{K \cdot a_0}{1 + K \cdot p_0}$$

Hence we obtain:

$$q = \frac{K \cdot p_0}{1 + K \cdot p_0} \cdot a_0 \qquad (4)$$

and in the case of one functioning part:

$$J = J_0 \cdot e^{-\frac{K \cdot a_0}{1 + K \cdot p_0} N} \qquad (5)$$

The equilibrium constant for the reaction of interaction of AM and DNA molecules was determined in a study by Permogorov and Lazurkin [6]. It has the value $K_o \approx 3 \cdot 10^4$ per mole of nitrogenous bases. However, in formula (5) it is necessary to use a value K related to 1 mole of guanine bases. Here it must be remembered that the AM joining one DNA strand also inhibits synthesis on the other strand [1]. Thus, it is necessary to take into account the number of the guanine-cytosine pairs in the DNA and, therefore, assume $K = K_o/\alpha$, where α is the relative content of guanine-cytosine pairs.

Formula (5) describes the process of inhibition of RNA synthesis in a cell-free system. A priori this process in a cell may behave differently. Nevertheless, it is interesting to determine to what degree the derived quantitative relations can be considered as at least a first approximation.

Method

The work was done on cells of Ehrlich's ascitic carcinoma and on cells of a transplanted CaVe culture. The incubation medium for the ascitic cells consisted of a single volume of Robinson's solution with glucose (9 mg/ml) and folic acid (0.72 μmole/ml), two volumes of water and 1½ volumes of ascitic fluid. In order to obtain a suspension of ascitic cells of different density, the initial suspension was diluted with an incubation medium to the necessary value. The CaVe cells were cultivated in matrasses in a 199 medium with the addition of a 20% bovine serum. For the experiment the cells were removed from the matrasses with trypsin and put into suspension. The incubation was at 37° and with constant shaking. The tagged precursor was C^{14}-uranyl (54.2 mcurie/g). Soviet-produced actomycin 2703 was used in the work.

After the necessary incubation time, the cells were cooled and washed three times in a 5% solution of $HClO_4$ and alcohol. The acid-insoluble precipitate was applied to a target and the residual radioactivity was determined using a gas-flow counter with a low background.

Results and Their Discussion

Below we describe experiments which in our opinion confirm the correctness of the considered model of inhibition of RNA synthesis.

If the bonding of an AM molecule also is described for the cell system by a simple reaction equation (3), and the simultaneously transpiring processes of growth suppression and stimulation in ~~repression and derepression of~~ different parts of the DNA exert a relatively /524 lesser influence on the course of RNA synthesis, the intensity of synthesis should vary with the value c in the same way, regardless of what factor is responsible for the variation of c. In other words, the dependence J = J(c) can be studied by varying c either by using different AM concentrations or by using different concentrations of nucleotides, that is, by varying the density of the studied cell suspensions.

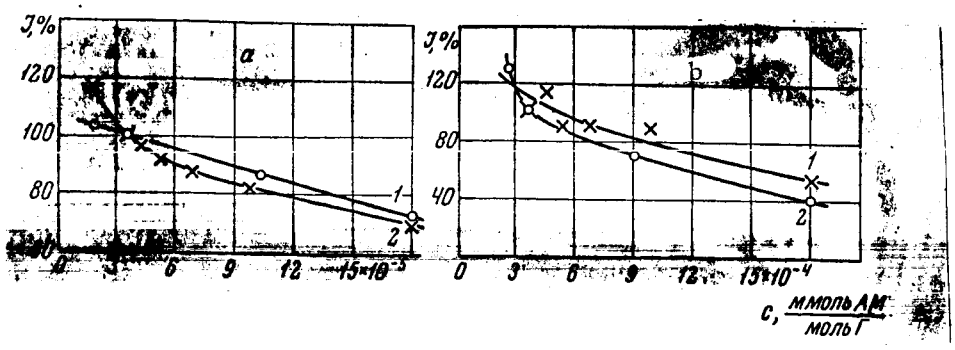


Figure 1. Change of the Residual Activity of RNA in Dependence on the Relative Concentration of Actinomycin.
Legend: 1 = density of the suspension changes; 2 = quantity of

Figure 1. (Continued). actinomycin changes; a = region of small concentrations; b = region of large concentrations; A = c, mmole AM/mole G.

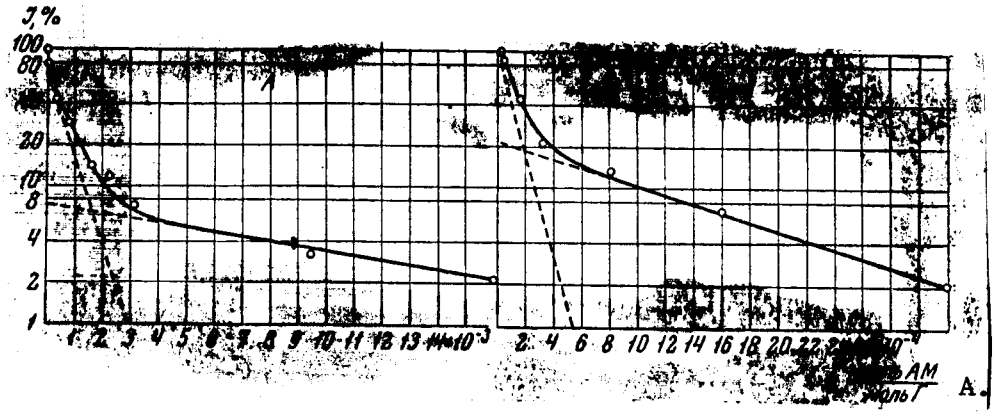


Figure 2. Inhibition of the RNA Synthesis in Ascites (A) and CaVe (B) Cells. The Scale is Semilogarithmic. The Dashed Line Represents the Exponents into Which the Experimental Curve is Broken Down.
Legend: A = c, mole AM/mole G.

Figure 1 shows the results of the corresponding experiments in two regions of values c. The curves 1 were obtained when the value c was changed due to the density of the suspension, whereas curves 2 were obtained when the changes c resulted from use of different AM concentrations. Residual activity is expressed in relative units; the curves cross at a single point. Despite the scatter of experimental points, on the whole we get the impression of a satisfactory agreement of both curves. True, in the case of a sharp decrease of the density of the suspension (which corresponds to large values c, exceeding by approximately five times the maximum values shown on the graph) the residual activity is sharply increased. The reasons for this circumstance could not be determined.

Another group of facts pertains to an analysis of the form of the dependence J(c). It goes without saying that under real conditions in the cell at the very same time many parts of the DNA can function and for practical purposes any decrement curve can easily be represented by a set of exponents. How- /525
ever, if a situation exists in which the RNA synthesis occurs in the cell at a high rate in only two or three groups of parts, having within a given combination a small scatter of numbers of guanine-cytosine pairs, then the curve J(c) is represented by the sum of two or three exponents. Figure 2A shows the results of experiments involving the inhibition of RNA synthesis in ascites cells; in Figure 2B -- in CaVe cells (the experimental data are represented semilogar-

ithmically). The graphs show that the points fall satisfactorily on curves resulting from addition of two exponents, which corresponds to the presence of two functioning parts (to be more precise, two groups of parts).

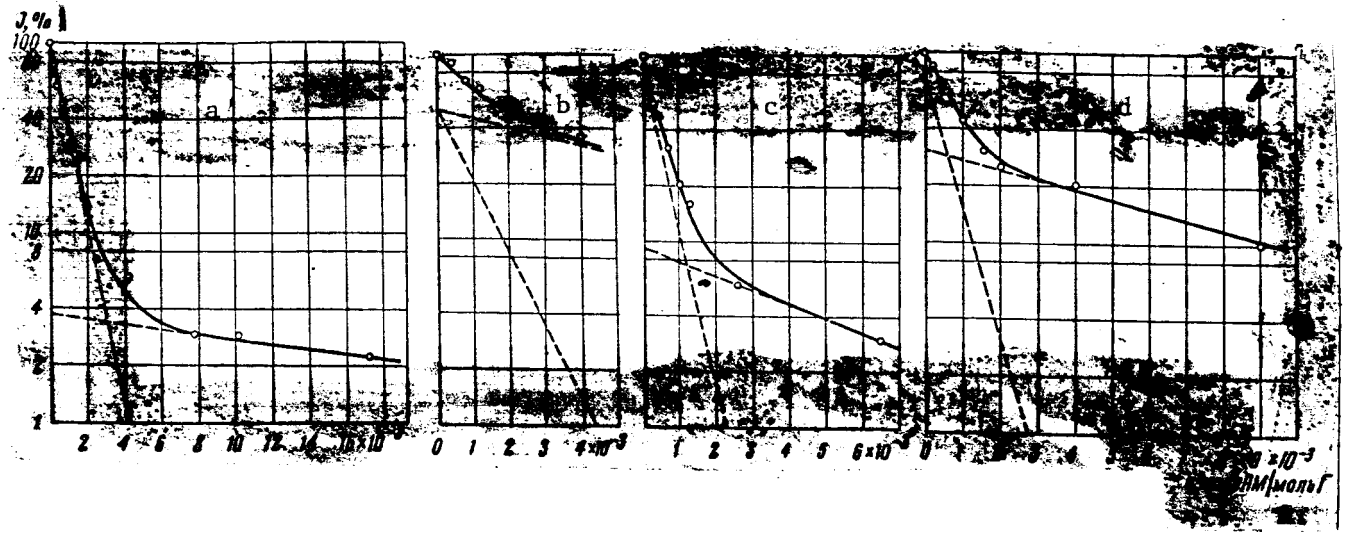


Figure 3. Inhibition of RNA Synthesis in a Cell-Free System, According to Data in the Literature. The Scale is Semilogarithmic. The DNA Template Used was DNA from the Following Objects: a) T2 Bacteriophage (17.5% Guanine [5]; b) Tetrahymena pyriformis (12.5% Guanine); c) Micrococcus lysodeicticus (37% Guanine); d) Calf Thymus (22% Guanine) [4]. Legend: A = c, mole AM/mole G.

We also processed experimental data on the AM inhibition of RNA synthesis in a cell-free system. These data were obtained by Permogorov, Prozorov, Shemyakin, Lazurkin and Khesin [5]. We also used data given in a study by Goldberg, et al. [4]. In this case as well (Figure 3), the graphs can be represented in the form of the sum of two exponents.

It is interesting to note that extremely similar results were obtained in experiments by Kahan, et al. [7], made in a cell-free system with the same components but with a lesser content of DNA template in the investigated sample. Thus, these data serve as a confirmation both of the considered model of inhibition as a whole and the circumstance that the value c can be varied by varying the quantity of DNA participating in the reaction, as was mentioned above.

Finally, using the slope of the semilogarithmic curve J(c), it is possible to estimate the value N in formula (5), that is, determine the number of guanine-cytosine pairs in a newly synthesized RNA molecule; it goes without saying, assuming that the value of the constant K and the value p_o are known.

The values K and p_o are computed in the following way. Assume that the number of cells present in one liter of the investigated suspension is x and the quantity of DNA in a cell of a particular type is β. The quantity of /526

DNA in one liter of suspension will be βx (the quantity of DNA in an open system is measured directly and is determined by the experimental conditions). The molar concentration of nitrogenous bases is equal to βx/M, where M is the mean molecular weight of one nitrogenous base (approximately 330). The sought-for molar concentration of the guanine bases now is determined from the equation:

$$p_0 = \frac{\beta x}{M} \cdot \frac{\alpha}{2}$$

where α is the relative number of guanine-cytosine pairs in the DNA of the investigated object. The coefficient α is found from independent experimental studies of the nucleotide composition of DNA.

As mentioned above, the value K is determined using the formula $K = K_0/\alpha$, where $K_0 = 3 \cdot 10^4$.

As an illustration we will show how the values K and p_0 were computed in experiments with ascites (for other objects these values are computed in a similar way). The density of the suspension used was 10^8 cells/ml, that is, $x = 10^{11}$ cells/liter. The quantity of DNA in one ascites cell is $\beta \simeq 6 \cdot 10^{-12}$g. The relative concentration of guanine-cytosine pairs for cells of the particular type is $\alpha = 0.44$. Hence $p_0 = 4 \cdot 10^{-4}$, and the value $K = 6.8 \cdot 10^4$.

ESTIMATE OF THE NUMBER OF GUANINE-CYTOSINE PAIRS IN THE FUNCTIONING PART

Object	Region of J = J(c) graph	Number of guanine-cytosine pairs
Ascites	1	1500
	2	100
CaVe	1	2000
	2	200
T2 Bacteriophage	1	1100
	2	100
Micrococcus lysodeicticus	1	1900
	2	170
Tetrahymena pyriformis	1	900
	2	120
Calf thymus	1	1600
	2	100

The results of the corresponding computations of the value N for the graphs shown in Figures 2 and 3 are given in the table. Region 1 of the graph corresponds to minimum relative AM concentrations, that is, to the initial part of the inhibition curve; region 2 corresponds to higher AM concentrations.

It should be emphasized that the slope of the curve can be used only for determining the number of guanine-cytosine pairs in a definite functioning part. The total number of nucleotides in a functioning part, and, therefore, the entire length and molecular weight of the synthesized RNA are dependent, in addition, on the value of the specificity factor of that ribonucleic acid which is synthesized in it.

Conclusions

On the basis of simple statistical concepts, a study has been made of the mechanism of inhibition of RNA synthesis in a cell-free system. It has been demonstrated experimentally that the derived quantitative relationships can be used also in the case of intact cells. An estimate is given for the number of guanine-cytosine pairs in the considered DNA section. Under the influence of AM there first will be an inhibition of those RNA molecules which have the greatest molecular weight and whose nucleotide composition includes a relatively large number of guanine-cytosine pairs, that is, high-molecular RNA of the guanine-cytosine type. This also can be attributed to the fact [8,9] that AM first inhibits synthesis of ribosomal RNA (high-molecular and guanine-cytosine type), and, second, inhibits the synthesis of messenger RNA (high-molecular, but the AU type) and, thereafter, inhibits the synthesis of transport RNA (low-molecular, but of the guanine-cytosine type).

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